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LABORATORY INVESTIGATION

Intrarenal hemodynamic alterations induced by anti-GBM antibody

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Intrarenal hemodynamic alterations induced by anti-GBM antibody. An isolated perfused kidney system (IPK) was used to study the direct intrarenal hemodynamic effects of binding of anti-glomerular–basement membrane (anti-GBM) antibody in the absence of all other circulating humoral and cellular inflammatory mediators. Control IPK's (perfused with Krebs–Henseleit buffered 5% albumin solution containing non-immune globulin) had a renal vascular resistance (RVR) mean ± SEM 3.10 ± 0.47 mm Hg/ml/min and a GFR mean ± SEM 0.63 ± 0.8 ml/min/g. Anti-GBM antibody administration raised RVR (4.83 ± 0.52 mm Hg/ml/min, $P < 0.01$) and lowered GFR (0.34 ± 0.04 ml/min/g, $P < 0.01$). Perfusate renin activity was higher after antibody administration (684 ± 87 ng AI/ml/hr compared with control 308 ± 42 ngAI/ml/hr, $P < 0.01$). Treatment with Sar^{1}Ala^{8}All (3×10^{-6}M) or captopril (10 mg/ml) attenuated antibody–induced vasoconstriction (RVR mm Hg/ml/min, Sara^{1}Ala^{8}All = 3.78 ± 0.13 captopril = 3.26 ± 0.12, both $P < 0.05$ compared with anti-GBM alone). Both inhibitors of the renin–angiotensin system (RAS) also aggrevated the decline in GFR seen after antibody administration (GFR ml/min/g, Sara^{1}Ala^{8}All = 0.24 ± 0.05, Captopril = 0.18 ± 0.03, both $P < 0.05$ compared with anti-GBM alone). These IPK studies demonstrate that anti-GBM antibody itself may directly induce intrarenal hemodynamic alterations in the absence of complement activation, neutrophil infiltration, neural influences or circulating vasoactive substances. The results from perfusate renin sampling and blockade of the RAS provide evidence that anti-GBM antibody deposition activates the intrarenal RAS and thereby induces significant hemodynamic alterations.

Anti-GBM antibody–induced glomerulonephritis (antiGBM-GN) is a widely studied model of immune renal injury [1–4]. The development of renal injury is typically monitored by assessing changes in glomerular permeability, using measurements of urinary protein excretion rates. The important contributions of hemodynamic alterations to the evolution of both immunological and non-immunological renal disease have recently been emphasized [5–7]. While renal injury in acute glomerulonephritis is acknowledged to involve alterations in renal hemodynamics [7–10], the mechanisms by which immunological stimuli induce these changes remain incompletely understood [10–13]. In studies of the renal hemodynamic changes occurring in glomerulonephritis in vivo there are difficulties in dissecting the relative influences of altered autonomic tone, altered levels of circulating vasoactive substances, and systemic hemodynamic changes on intrarenal hemodynamics [13]. Immune injury may follow antibody deposition through a variety of potential effector mechanisms. The potential for complement activation and inflammatory cell infiltration to produce hemodynamic change has been demonstrated in experimental GN [10, 11]. However, in a variety of experimental and human nephritides, inflammatory cells are absent and complement is only sparsely or inconsistently deposited [14–15], clearly indicating that complement and inflammatory cell independent mechanisms of injury exist. In whole animal studies it is difficult to discern the individual contribution of each mediator towards the production of renal injury [13]. An IPK system facilitates administration of single putative mediators in an environment which is devoid of all other circulating humoral and cellular mediators of injury. The isolated organ system is also not subject to either neural or systemic hemodynamic influences. We therefore chose to use an IPK system to assess the direct effects of antibody binding on intrarenal hemodynamics. This system allowed the dissection of the direct effects of antibody itself on the kidney from indirect effects due to activation of regional and systemic injury mediation systems.

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Methods

Perfusion technique

The perfusion apparatus used in these experiments was similar to that described by Bowman and Maack [16] and modified by Nakane et al [17] and Epstein et al [18]. The apparatus consisted of a central glass reservoir containing the perfusate which was recirculated by a roller peristaltic pump (Watson–Marlow, London, UK). The perfusate traversed two bubble traps and two 8 μ filters (Millipore, Bedford, Massachusetts, USA) before being gassed with a mixture of 95% O_2/5% CO_2 and delivered to the glass arterial perfusion cannula (P.A. Brooks, Ducklington, UK).

Male Sprague–Dawley (SD) rats (300 to 450 g) were anaesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg). A laparotomy was performed after injection of 1 ml of 10% mannitol and 500 IU sodium heparin (Commonwealth Serum Laboratories, Melbourne, Australia) into the left femoral vein. The right ureter was cannulated with polyethylene tubing (PE-10, Intramedic, Parsippany, New Jersey, USA). The right renal artery was cannulated via the superior mesenteric artery and the kidney perfused during en-bloc dissection. After removal the kidney surface was rinsed with phosphate buffered saline (PBS) and 20 to 40 ml of perfusate flushed through the

renal vasculature before transferring the kidney to a support above the central reservoir. The renal vein was not cannulated. The kidney was allowed to equilibrate for 10 minutes. The perfusate flow rate was >20 ml/min at a cannula tip pressure <120 mm Hg by the end of this equilibration period. The kidney was covered with a paraffin film (Nescofilm, Bando Chemical Ind. Ltd., Kobe, Japan) and perfused for periods up to 150 minutes. Samples of perfusate were taken at 10 minute intervals and urine was collected over 20 minute intervals. All samples were stored at −20°C prior to assay. Globulin fractions were added to the perfusate reservoir at the end of the equilibration period (t_o). After the end of the equilibration period no adjustment to perfusion pressure and/or flow were made in either experimental or control perfusions.

The IPK perfusate consisted of a buffered albumin solution (BAS), a Krebs–Henseleit buffer containing 5.0 g/liter bovine serum albumin (Sigma Chemical Co., St. Louis, Missouri, USA) with added creatinine 10 to 20 mg/liter), glucose (5 mM/liter) and amino acids [18] was prepared. It was prefiltered and then passed through a 0.22 μ filter (Millipore) before use within eight hours of preparation. Renin assays were performed by the Laboratory of Professor Colin I. Johnston using previously described methods [19].

Perfusate containing normal sheep globulin (1 mg/ml) or anti-GBM antibody (1 mg/ml) was assayed for angiotensinogen activity by substituting perfusate for plasma renin substrate in a previously described renin assay (assay sensitivity 0.2 ng AI/ml).

The renin source used in this assay was hog renin (1 unit). Normal rat plasma incubated under identical conditions in this assay generates 441.4 ± 14.9 ng AI/ml. Sheep renin substrate generates 1,109.1 ± 40.5 ng AI/ml. The perfusate did not interfere with the detection of AI in this assay over the 0.2 to 20 ng/ml range tested. Incubation of perfusate with hog renin (1 unit) resulted in no measured AI generation at 0, ½, 1, 2, 4 and 6 hours. The sensitivity of this assay is 0.2 ng/ml. The perfusate thus contained less than 0.05% of the angiotensinogen activity of normal rat plasma.

Assessment of renal function

Protein excretion. This was measured in duplicate samples by the Comassie Brilliant Blue dye binding assay [20].

Glomerular filtration rate (GFR). This was measured in duplicate samples by both the inulin clearance and creatinine clearance techniques, which gave identical results.

Renal vascular resistance (RVR). Perfusate pressure was measured proximal to the arterial cannula with a calibrated anaeroid manometer. The cannula tip pressures were derived by subtracting from the measured pressure the pressure drop known to occur across a particular arterial cannula (of known resistance at a given flow). Perfusate flow was measured by a drop counter above the central reservoir. Then, resistance = cannula tip pressure (mm Hg)/perfusate flow (ml/min).

Sodium (Na) and creatinine (Cr) estimates. Sodium was determined using a flame photometer (Beckman Instruments, Palo Alto, California, USA). Creatinine was determined using a colorimetric automated analyser (Beckman). The fractional reabsorption of sodium (FR_{Na}) was calculated by

$$FR_{Na}\ (\%) = 1 - \frac{UNa.V}{GFR.P_{Na}} \times 100$$

where U_{Na} = urine Na (mM/liter), V = urine flow (ml/min) and P_{Na} = perfusate Na (mM/liter).

Renin activity. Perfusate renin activity was determined by radioimmunoassay for angiotensin I after incubation with rat plasma renin substrate for 30 and 60 minutes and then expressed as the amount of angiotensin I formed during one hour of incubation [19].

Regional perfusate flow. Microspheres were used to assess the distribution of perfusate flow between outer and inner renal cortex as previously described [21]. Radioactive scandium (^{46}Sc) and cerium (^{141}Ce) labelled microspheres of 15 μ diameter (New England Nuclear, Boston, Massachusetts, USA) suspended in 10% dextran and 0.1% Tween 80 were prepared for injection by sonicating for 10 minutes. Ten thousand microspheres suspended in 10 μl were mixed with 40 μl of perfusate and injected in to the arterial perfusion cannula. ^{141}Ce-labelled microspheres were injected during the control period and the ^{46}Sc-labelled microspheres injected 45 minutes after addition of antibody. The cortex of the middle third of the kidney was carefully dissected into inner and outer cortex, weighed and from their respective radioactivities, and regional flow was determined [21].

Histological assessment

Tissue for immunofluorescence was taken after completion of perfusion, snap–frozen in liquid nitrogen and stored at −70°C. Two–micron frozen sections were cut on a cryostat and then stained with antisera to sheep globulin (IgG, Dakopatts, Sweden), Sheep C3 (research Plus Inc., Bayonne, New Jersey, USA) rat IgG (Sigma), and rat C3 (United States Biochemical Corporation, Cleveland, Ohio, USA). The sections were examined under a Leitz Othlux II UV microscope (Leitz, Heerbrugg, Switzerland).

Antibodies

Normal sheep globulin (NSG). Normal sheep serum was extensively absorbed against SD rat red blood cells (RBC's) and plasma. A globulin fraction was obtained by precipitation with saturated ammonium sulphate. The precipitate was resuspended in phosphate buffered saline (PBS) and passed through a 0.22 μ filter.

Sheep-anti-rat GBM globulin (anti-GBM antibody). Sheep were repeatedly immunized with particulate SD GBM as previously described [22]. When a high titer of anti-GBM antibody was established, as determined by radioimmunoassay (RIA) the sheep was sacrificed. The sera was absorbed and a globulin fraction prepared as for NSG.

Quantitation of antibody binding. Paired–label globulin binding studies were performed for every perfusion after trace radiolabelling the globulin with NSG or antiGBM-Ab with ^{131}Iodine or ^{125}Iodine using the Chloramine-T technique [23]. Trace labelled globulins were added at the end of the IPK equilibration period to a known volume of perfusate, and perfusate was sampled after thorough mixing had occurred for determination of its specific activity. At the desired time the kidney was removed from the perfusion apparatus, trimmed of

Table 1. Control perfused kidneys

Additions to IPK perfusate			Parameter	Time, *min* 20	40	60	80	100	120
Control IPK function									
Drug	NSG	*N*	Protein excret *mg/min*	0.253 ± .047	0.174 ± .031	0.155 ± .028	0.217 ± .065	0.231 ± .046	0.309 ± .048
Nil	1 mg/ml	13	GFR *ml/min/g*	0.68 ± .09	0.67 ± .08	0.63 ± .06	0.60 ± .09	0.62 ± .08	0.57 ± .08
			RVR *mm Hg/ml/min*	3.23 ± .40	2.72 ± .27	2.82 ± .20	2.83 ± .30	3.27 ± .34	3.90 ± .47
			Fractional reabsorption of sodium (%) ± SEM	90 ± 2.4	91 ± 1.8	90 ± 2.8	91 ± 1.8	90 ± 2.4	90 ± 2.4
Additions to IPK perfusate									
Drug	NSG	*N*	Protein excretion *mg/min*	0.198 ± .115	0.221 ± .119	0.197 ± .084	0.183 ± .104	0.178 ± .081	0.188 ± .101
Captopril	1 mg/ml	6	GFR *ml/min/g*	0.67 ± 0.06	0.69 ± 0.08	0.65 ± 0.04	0.63 ± 0.05	0.61 ± 0.04	0.61 ± 0.04
			RVR *mm Hg/ml/min*	3.10 ± .42	2.89 ± .51	2.70 ± .49	2.76 ± .55	2.80 ± .61	2.82 ± .47
			Protein excretion *mg/min*	0.213 ± .114	0.225 ± .097	0.210 ± .105	0.173 ± .114	0.185 ± .099	0.179 ± .100
Saralasin	1 mg/ml	6	GFR *ml/min/g*	0.66 ± 0.04	0.67 ± 0.05	0.64 ± 0.06	0.61 ± 0.05	0.59 ± 0.04	0.58 ± 0.06
			RVR *mm Hg/ml/min*	3.15 ± .51	3.01 ± .45	2.78 ± .35	2.80 ± .42	2.81 ± .39	2.96 ± .41

capsule, excess connective tissue and fat and the renal cortex dissected free. The cortex was then weighed before being homogenized in a glass tube homogenizer. The homogenate was repeatedly washed in 50 ml of PBS until the supernatant was clear. Specific kidney fixed antibody levels (KFA) were assessed as previously described [23] and expressed as μg antibody globulin bound per gram of renal cortex (μg/g). The perfusions continued for a maximum of 150 minutes, with protein excretion, GFR, RVR and FR_{Na} calculated for each 20 minutes interval to quantitate renal function. Control (NSG) perfusions contained concentrations of globulin equivalent to the maximal antibody concentrations achieved (1 mg/ml). The ability of corticosteroids, antihistamines, renin–angiotensin inhibitors and prostaglandin synthetase inhibition to modify the effects of anti-GBM-Ab administration was assessed by perfusing with methylprednisolone (400 mg/ml), promethazine (500 mg/ml) captopril (10 mg/ml), $Sara^1Ala^8All$ (3×10^{-6}M) and indomethacin (1 mg/ml), added during the equilibration period.

Statistical methods

Data is expressed as mean ± SEM. Results of GFR and RVR for sequential 20 minute intervals are shown in the Tables. In the text all data is presented as the overall mean ± SEM for the 120 minute study period. Significance values for comparisons between grouped data were determined using the one–way analysis of variance.

Results

BAS perfused controls

Isolated rat kidneys, perfused with a buffered albumin solution with added normal sheep globulin (1 mg/ml) and stable function for the 120 minutes of the study (Table 1). Urinary protein excretion ranged from 0.155 to 0.309 mg/min (mean 0.223 ± 0.44 mg/min), GFR ranged from 0.57 to 0.68 ml/min/g (mean 0.63 ± 0.08 ml/min/g), RVR ranged from 2.72 to 3.90 mm Hg/ml/min (mean 3.10 ± 0.47 mm Hg/ml/min) and FR_{Na} was > 90%. Perfusate samples assayed from control (N = 6) kidneys demonstrated a basal renin activity of 308 ± 24 ngAI/ml/hr. Perfusate containing NSG or antiGBM antibody had no demonstrable angiotensinogen activity.

Globulin/antibody binding studies

Antibodies bound to the perfused kidney in a dose related fashion. Anti-GBM-Ab was shown to localize to the GBM with linear staining on IF, with no significant staining of the vascular endothelium or renal tubular basement membrane. No other immune reactants were deposited in glomeruli by IF examination.

Effects of anti-GBM antibody alone

At KFA 385 μg/g. Anti-GBM-Ab binding at this level produced marked renal hemodynamic change, as indicated by an increased RVR (4.83 ± 0.52 mm Hg/ml/min, $P < 0.01$) and a decreased GFR (0.34 ± 0.04 ml/min/g, $P < 0.01$) (Table 2, Fig. 1). Renal protein excretion also increased (2.009 ± 0.681 mg/min, $P < 0.01$), as discussed in a companion study [24]. This level of antibody binding increased perfusate renin activity significantly (684 ± 87 ng/Al/ml/hr $P < 0.01$) (Fig. 1).

At KFA 183 μg/g & 89 μg/g. Antibody binding at these KFA levels was associated with no change in the rate of protein excretion, although a fall in GFR (0.41 ± 0.08 ml/min/g) and rise in RVR (4.72 ± 0.43 mm Hg/ml/min) still occurred, both of which were significant ($P < 0.05$) (Table 2). These hemodynamic changes were quantitatively similar to those occurring at the 385 μg/g dose, as was the increase in perfusate renin activity (595 ± 63 ngAI/ml/hr, $P < 0.05$).

At KFA 89 μg/g. At this level of binding anti-GBM-Ab produced no discernible alteration in protein excretion (0.222 to 0.003 mg/min), GFR (0.66 ± 0.04 ml/min/g) or RVR (3.85 ± 1.24

Table 2. Effects of antibody alone on IPK function

AntiGBM antibody KFA ± SEM	*N*	Parameter\time, *min* ± SEM	20	40	60	80	100	120
385 μg/g ± 16	9	Protein excretion *mg/min*	0.487 ± .124	1.058 ± .372	1.214 ± .380	1.668 ± .441	2.502 ± .500	5.126 ± 1.618
		GFR *ml/min/g*	0.49 ± .03	0.40 ± .04	0.34 ± .04	0.27 ± .05	0.31 ± .05	0.23 ± .04
		RVR *mm Hg/ml/min*	3.75 ± .27	4.43 ± .73	4.20 ± .56	4.94 ± .56	5.26 ± .64	6.41 ± .52
183 μg/g ± 8	6	Protein excretion *mg/min*	0.337 ± .068	0.237 ± .069	0.248 ± .064	0.326 ± .087	0.316 ± .112	0.374 ± .001
		GFR *ml/min/g*	0.51 ± .04	0.47 ± .06	0.42 ± .05	0.41 ± .05	0.38 ± .04	0.28 ± .04
		RVR *mm Hg/ml/min*	4.04 ± .41	3.92 ± .43	4.91 ± .69	5.12 ± .63	5.06 ± .67	5.25 ± .55
89 μg/g ± 4	4	Protein excretion *mg/min*	0.200 ± .024	0.200 ± .183	0.213 ± .106	0.209 ± .021	0.236 ± .076	0.274 ± .089
		GFR *ml/min/g*	0.69 ± .03	0.68 ± .02	0.68 ± .02	0.65 ± .02	0.63 ± .03	0.64 ± .02
		RVR *mm Hg/ml/min*	3.80 ± 1.21	3.75 ± 1.17	3.68 ± 1.05	3.79 ± 1.23	3.98 ± 1.61	4.12 ± 1.34

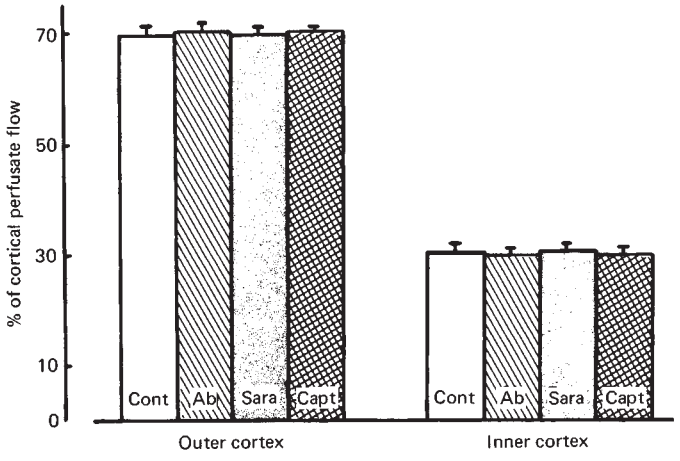

Fig. 1. *The administration of antibody, saralasin and captopril did not significantly alter regional cortical perfusate flow from control values.*

mm Hg/ml/sec) (Table 2). Perfusate renin activity was not significantly different from control values (334 ± 35 ngAI/ml/hr).

Pharmacological studies in IPK's given anti-GBM Ab. In IPK perfusions performed with the addition of anti-GBM Ab (385 μg/g) there was no significant amelioration of injury in the presence of corticosteroids, (prednisolone), antihistamines (promethazine) or a prostaglandin–synthetase inhibitor (indomethacin) (Table 3). However inhibition of the renin–angiotensin system using an AII receptor antagonist (Sara^{1}Ala^{8}AII) and an angiotensin–converting enzyme inhibitor (captopril) reduced antibody induced vasoconstriction (RVR mm Hg/ml/min Sara^{1}Ala^{8}AII 3.78 ± 0.13, captopril 3.26 ± 0.12, both $P < 0.05$ of antibody alone). This reduction in vasoconstriction resulted in both a fall in perfusion pressure and a rise in perfusate flow rates when compared with antibody treated preparations. RAS inhibition also exaggerated the fall in GFR produced by antibody (GFR ml/min/g Sara^{1}Ala^{8}AII 0.24 ± 0.05, captopril 0.18 ± 0.03, both $P < 0.05$ cf antibody alone). The hemodynamic (that is, GFR and RVR) changes induced by captopril treatment were more marked than those with saralasin ($P < 0.05$). None of the inhibitors significantly altered protein excretion rates or the quantity of antibody deposited in glomeruli (Table 3). The administration of captopril or saralasin to control (NSG) IPK's resulted in no significant alterations in IPK function (Table 1).

Regional perfusate flow. Control perfusions had 69.7 ± 1.9% of total cortical perfusate flow directed to the superficial cortex and 30.3 ± 1.9% to the deep cortex (Fig. 2). Antibody administration Sara^{1}Ala^{8}AII treatment and captopril treatment did not significantly alter the distribution of cortical perfusate flow as assessed by microsphere distribution.

Discussion

The isolated perfused rat kidney was initially developed by Weiss for the study of renal hemodynamics [25]. It allows assessment of the direct effects of agents on renal hemodynamics, eliminating extrarenal influences such as the sympathetic nervous system, systemic hemodynamic alterations and circulating vasoconstrictor substances [24–26]. The IPK also facilitates the study of individual inflammatory mediators by allowing their sequential repletion to the circulating perfusate [27–29], avoiding many of the difficulties inherent to in-vivo mediator depletion studies. Finally, the IPK is an ideal system with which to study renal vasoconstriction [25, 26, 30], as it is a system in which accurate pressure/flow data is easily obtained, thus facilitating detection and quantitation of increases in vascular tone.

The current study clearly demonstrates that anti-GBM antibody itself can directly induce renal hemodynamic changes. Furthermore, the IPK model has allowed dissection of the mechanism of action of antibody induced injury within the organ of interest. Whole animal studies have suggested that the hemodynamic changes following anti-GBM antibody administration are largely due to glomerular complement activation and neutrophil infiltration [11, 12]. However in mediator depletion studies, in vivo antibody induces hemodynamic alterations despite apparent blockade of the complement system and prevention of glomerular neutrophil infiltration [12]. In whole animal studies, many potential circulating substances could account for the hemodynamic changes induced by antibody deposition in complement and neutrophil depleted animals. The IPK allows evaluation of the effects of individual mediators on intrarenal hemodynamics, and clearly demonstrates that antibody can directly induce intrarenal hemodynamic changes in the absence of any other circulating factors. In the current study, in the absence of other inflammatory mediator systems (that is, complement, neutrophils), a relatively high level of

Table 3. Attempted blockade of the effects of anti-GBM antibody on IPK function

Drug added to the IPK perfusate	*N*	Parameter	Time, *min* 20	40	60	80	100	120
Methyl prednisolone	6	Protein excretion *mg/min*	0.485 ± .114	0.815 ± .164	0.217 ± .224	1.558 ± .277	2.095 ± .299	2.105 ± .319
		GFR *ml/min/g*	0.60 ± .01	0.53 ± .03	0.47 ± .03	0.42 ± .03	0.37 ± .05	0.34 ± .06
		RVR *mm Hg/ml/min*	3.74 ± .33	3.89 ± .42	3.89 ± .58	4.08 ± .61	4.43 ± .60	5.92 ± .76
Promethazine hydrochloride	4	Protein excretion *mg/min*	0.391 ± .100	0.6444 ± .297	0.653 ± .139	1.220 ± .320	1.322 ± .508	1.999 ± .422
		GFR *ml/min/g*	0.49 ± .05	0.47 ± .06	0.43 ± .01	0.39 ± .02	0.28 ± .02	0.22 ± .04
		RVR *mm Hg/ml/min*	3.65 ± .19	3.64 ± .36	4.07 ± .41	4.98 ± .64	5.61 ± .78	5.65 ± .80
Indomethacin	4	Protein excretion	0.440 ± .130	0.608 ± .120	0.154 ± .245	1.192 ± .315	1.593 ± .209	2.132 ± .352
		GFR *ml/min/g*	0.56 ± .08	0.51 ± .11	0.47 ± .13	0.42 ± .14	0.40 ± .12	0.37 ± .12
		RVR *mm Hg/ml/min*	4.36 ± .23	4.60 ± .66	5.27 ± .76	6.62 ± .72	6.40 ± .58	6.71 ± .50
Captopril	6	Protein excretion *mg/min*	0.248 ± .102	0.458 ± .183	1.221 ± .853	1.426 ± .898	1.985 ± .735	2.024 ± .857
		GFR *ml/min/g*	0.41 ± 0.04	0.20 ± 0.03	0.12 ± 0.05	0.15 ± 0.04	0.09 ± 0.03	0.08 ± 0.03
		RVR *mm Hg/ml/min*	3.28 ± .16	2.78 ± .32	2.82 ± .41	2.88 ± .36	3.25 ± .45	3.88 ± .49
Saralasin	6	Protein excretion *mg/min*	0.259 ± .085	0.685 ± .342	1.059 ± .305	1.936 ± .480	2.304 ± .887	2.461 ± .914
		GFR *ml/min/g*	0.45 ± 0.05	0.28 ± 0.03	0.20 ± 0.02	0.20 ± 0.02	0.18 ± 0.03	0.16 ± 0.02
		RVR *mm Hg/ml/min*	3.37 ± .09	3.24 ± .10	3.49 ± .15	3.75 ± .15	4.20 ± .13	4.64 ± .13

The addition of corticosteroid, promethazine or indomethacin to the IPK perfusate did not significantly alter the injury produced by anti-GBM antibody. Saralasin and captopril both significantly attenuated the hemodynamic injury induced by anti-GBM antibody binding at 380 μg 1 g KFA.

bound antibody was required to induce hemodynamic changes. In vivo, multiple mediator systems contribute to immune renal injury. The relative importance of antibody itself in immune renal injury will vary in proportion to the quantity of glomerular bound immunoglobulin [31].

Antibody–induced hemodynamic abnormalities may be produced by two alternative mechanisms. Anti-GBM antibody binding may exert its hemodynamic effects directly by interaction with vascular structures or indirectly by promoting the release of mediator substances from intrinsic renal parenchymal cells. The antibody used did not bind to vascular structures, making it unlikely that direct antigen–antibody interactions provoked the observed hemodynamic changes. The alternative mechanism is that of anti-GBM antibody–stimulated local release of mediator substances. The ability of intrinsic renal cells to elaborate inflammatory/vasoactive mediators has recently been demonstrated by Pirotsky et al (29), highlighting the possible contribution of such mechanisms to the production of renal injury.

This hypothesis led us to investigate the effects of pretreatment of our IPK preparation with antagonists of several putative mediators. There was no effect of pretreatment with a prostaglandin synthetase inhibitor (indomethacin), a histamine antagonist (phenergan), or corticosteroids (methyl prednisolone). However pretreatment with an angiotensin–converting–enzyme (ACE) inhibitor (captopril) or a competitive antagonist of angiotensin II (Sara^{1}Ala^{8}AII) markedly attenuated antibody induced vasoconstriction and augmented the decline in GFR seen after antibody administration. The changes which occurred with captopril pretreatment were quantitatively more pronounced than those with Sara^{1}Ala^{8}AII. This may reflect an additional modifying effect on the renal vasculature by other vasoactive substances produced by captopril therapy such as, bradykinin, prostaglandins [32]. Alternatively, it may reflect a reduced availability of Sara^{1}Ala^{8}AII at intrarenal AII binding sites [33].

Perfusate renin activity in control IPK preparations was similar to previously reported values [33–35], with a significant increase occurring after antibody administration. The augmented renin release which followed antibody administration is likely to be a specific consequence of antibody binding to the glomerular basement membrane, as the hemodynamic changes per se would be expected to lead to a markedly decreased, rather than an increased, perfusate renin activity [35]. The mechanism/s by which antibody binding promotes intrarenal renin release remain speculative. It is possible that glomerular immunoglobulin deposition alters the physical environs of the juxtaglomerular cell (such as, electrostatic charge:GBM conformation) and directly stimulates renin release. Alternatively, the release of a "second messenger" from intrinsic glomerular cells could indirectly promote renin release from juxtaglomerular cells. As there is no source of renin substrate (angiotensinogen) in the IPK perfusate, the vascular responses following antibody administration, and their modification by inhibition of the renin–angiotensin system (RAS), must reflect changes in the activity of the intrarenal RAS. The importance of the intrarenal RAS in a variety of physiological and pathophysiological circumstances is increasingly recognized [32, 37–39]. Previous in vivo studies have failed to define a role for AII mediation of the hemodynamic alterations in anti-GBM-GN [10]. The current studies utilized a dose of Sara^{1}Ala^{8}AII which was adequate to inhibit the intrarenal RAS [32]. Previous in vivo studies have used doses of Sara^{1}Ala^{8}AII which, while sufficient to inhibit the RAS intravascularly, would be expected to have little effect on the intrarenal RAS [10, 33], thus a contribution of the intrarenal RAS to the hemodynamic changes in anti-GBM-GN would have remained unrecognized. It would be exceedingly difficult to interpret renal hemodynamic changs occurring in whole animal studies using large doses of AII receptor antagonists or converting enzyme inhibitors, as these agents exert pronounced effects on systemic hemodynamics, in addition to their effects on the intrarenal RAS. In the current study neither captopril nor

Sara[1]Ala[8]AII significantly altered the relative distribution of cortical perfusion following anti-GBM antibody administration and there was a marked fall in GFR, despite vasodilatation, after RAS blockade in antibody treated kidneys. These data suggest that the prime site of action of the antibody–stimulated increase in intrarenal AII was on the efferent arteriole (a finding consistent with data from other investigators [19, 32–39]), and that antibody binding reduced the glomerular capillary ultrafiltration coefficient (Kf). Thus, while the fall in Kf led to a reduction in GFR, the activation of the RAS 'protected' GFR by constriction of the efferent arteriole and elevation of the mean transcapillary hydraulic pressure difference (ΔP). Blockade of the RAS in antibody treated preparations prevented this 'protective' elevation of ΔP, resulting in exacerbation of the antibody–induced decline in GFR simultaneous with reduction in total RVR.

As we have reported elsewhere [24] antibody administration in the IPK is also associated with the development of heavy proteinuria and loss of the negative charge barrier on the glomerular filter. The magnitude of the increase in protein excretion is far in excess of that which could be explained in terms of hemodynamic changes alone. There are different antibody binding thresholds for the development of hemodynamic alterations and for the development of altered glomerular permselectivity. Furthermore, RAS blockade with restoration of near–normal perfusate flow does not protect against the development of proteinuria. In combination these data suggest that there are independent mechanisms which mediate the changes in renal hemodynamics and the altered glomerular permselectivity following glomerular antibody deposition.

Summary

Studies in the isolated perfused kidney have demonstrated that, in addition to its role in activating complement and recruiting other circulating mediators of injury, anti-GBM antibody can itself significantly alter renal hemodynamics. Antibody administration provoked an increased intrarenal renin release, with a significant proportion of the antibody induced hemodynamic change due to the enhanced intrarenal production of angiotensin II. This study demonstrates the potential for a direct intrarenal hemodynamic effect from the glomerular fixation of antibody. Such direct antibody–induced injury may contribute to the renal hemodynamic changes which occur in antibody–associated experimental and human renal disease.

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